QUANTITY OF 2,6-DICHLOROPHENOL IN AND ITS EFFECTS ON THE SEXES OF TWO SPECIES OF *Amblyomma* TICKS (*A. americanum* (LINNAEUS) AND *A. maculatum* KOCH)

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David Kellum

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David Kellum, son of Jimmie Lee and Lillie B. (Stephens) Kellum, was born in Opelika, Alabama on April 23, 1948. He attended Carver Elementary School and J. W. Darden High School. Upon graduating from Darden High School in June, 1966, he entered Talladega College in Talladega, Alabama. He entered Auburn University in January, 1968. He received the Bachelor of Science in March, 1972. His major was Zoology. After working as a research assistant at Auburn University for 6 months, he entered Auburn University and began work toward the Master of Science in September, 1972. He married Jacqueline L., daughter of Marion and Doris (Watkins) Burks, in August, 1972.

THESIS ABSTRACT

QUANTITY OF 2,6-DICHLOROPHENOL IN AND ITS EFFECTS ON THE SEXES OF TWO SPECIES OF *Amblyomma* TICKS (*A. americanum* (LINNAEUS) AND *A. maculatum* KOCH)

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An unusual natural compound in land animals, 2,6-dichlorophenol, was identified by Berger in 1972 as a sex pheromone of the female lone star tick, *Amblyomma americanum* (Linnaeus). Only traces of the pheromone were found in lone star males. The same chemical was also identified as a substantial component of both sexes of a closely related species, the Gulf Coast tick, *Amblyomma maculatum* Koch. The presence of this compound in both sexes of Gulf Coast ticks might explain a reported difference in the mating behavior of this tick from that of the lone star tick.

Analyses have verified these differences in 2,6-dichlorophenol content. The chemical is not present in the ticks at the time of emergence from nymph to adult, but occurs in steadily increasing amounts for 2-3 weeks. There is an apparent decrease in the amount of the phenol in unfed ticks after about 4 weeks.

Bioassays indicated that males of both species of the ticks are attracted to the compound. Only marginal evidence of attraction was indicated with females.

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I. INTRODUCTION

Ticks are important blood-sucking ectoparasites of animals and vectors of a wide range of bacterial, viral, rickettsial and protozoal diseases in all parts of the world. They are second only to the mosquitoes as transmitters of human diseases. The mere list of diseases is formidable and the effect of the diseases on the livestock industry is equally great. Some disease agents are important because of their lethal or debilitating effect on the host; others because livestock may act as reservoirs of infection to human beings. Some of the disease agents may also be transmitted by vectors other than ticks or by mechanical means from one host to another.

The total effect of ticks on the livestock industry of the world, whether by damage as parasites, transmitters of toxins, or vectors of disease, is incalculable. The cost to individuals and governments of preventing tick damage and tick-borne disease is very great.

The reduction or eradication of ticks and tick-borne diseases demands thorough knowledge of the biology and behavior of the ticks. The control of these pests has largely depended on toxic chemical agents. The recent concern over the harmful effects of the toxic chemicals has directed attention to other means of controlling these pests. Some believe that certain chemicals (pheromones) produced by an organism itself might be used to control or to adversely affect the behavior of that organism.

Sex pheromones or sex attractants are chemicals usually released by members of one sex of a species to stimulate behavioral responses in members of the opposite sex. They are important, if not essential, links in the process by which the sexes locate each other for mating. Although odors released by female insects are usually produced for the purpose of attracting males from a distance, they may also serve to sexually excite the male before copulation and to evoke a courtship response. Sexual odors released by males are used primarily for the purpose of sexually exciting the female, making her more receptive to the male's advances (aphrodisiacs). However, species are known in which males produce distance attractants for the female (Jacobson, 1972).

Sex pheromones have been demonstrated in various other organisms, including algae, nematodes, spiders, crustaceans, fishes, and mammals such as dogs, cattle, deer, mice, hamsters, and primates (Jacobson, 1972). The first demonstration of a sex pheromone in an arthropod other than insects was by Ryan (1966). He reported the presence of a water-soluble sex pheromone in the urine of females of the large crab, Portunus sanguinolintus (Herhst). It seemed reasonable to suspect that ticks also possess pheromones which could influence mating and affect feeding behavior since it had been reported that males of the lone star tick (Sacktor et al., 1948) and the Gulf Coast tick (Hixson, 1940) seek the females of their respective species in order to mate with them. Berger et al. (1971) demonstrated the presence of a substance in female hard ticks which stimulates behavioral responses in male ticks which they believed were characteristic of those exhibited in mating. This substance was also found in male ticks but only in trace amounts. Chemical analyses established that the compound is a phenol, 2,6-dichlorophenol (Berger, 1972). The question arose whether the chemical was a natural constituent of the ticks, since chlorinated organic compounds were not thought to occur in land animals. Although chlorinated chemicals and other organic compounds are widely distributed in the environment, it does not appear that the ticks acquire the chlorinated phenol from an external source (Berger, 1972). Later work by Berger (1974) has established that the compound is synthesized by the ticks. The bioassay used by Berger (1972) only showed that the responses elicited by the chemical are those of an attractant. No research on the effect of the chemical on the behavior of both sexes was done.

Gladney (1971) in studies of the mating behavior of Gulf Coast ticks concluded that the females seek the males for mating. His findings contradict those of Berger et al. (1971) in which the mating activities of the Gulf Coast tick and a closely related species, the lone star tick, were studied. The latter authors reported that the males of both species seek the females for mating purposes.

The phenol content of a particular tick would be expected to have a significant influence on the reactions of other ticks of the same species coming in contact with this individual. Therefore, studies were conducted to attempt to relate analyses of phenol content of the individual sexes of both species with observations of the behavioral responses of the ticks to the chemical. It was hoped these species might resolve the disputed difference in mating behavior of the 2 species of ticks.

II. REVIEW OF LITERATURE

Life History of the Lone Star and Gulf Coast Ticks

Lone Star Tick

The lone star tick, Amblyomma americanum (Linneaus, 1758) is one of the most economically and medically important species in the United States. It is widely distributed in the southern and eastern United States (Cooley and Kohls, 1944). The lone star tick is a non-specific parasite and feeds on a wide range of mammals and birds (Cooley and Kohls, 1944; Bishopp and Trembley, 1945). As a parasite it is important because it attacks man in all stages, and its unusually long mouth parts are deeply embedded in the host and often broken off when the tick is removed, thus predisposing the host to secondary infections and persistent and severe irritation (Bishopp and Trembley, 1945). Its great abundance on various wild and domestic hosts causes much annoyance and loss of blood. As a disease vector it assumes importance since it is capable of transmitting a large number of diseases which include tularemia, Rocky Mountain spotted fever, and Colorado tick fever among others (Arthur, 1962). In areas where a high density of ticks occurs outdoor recreation and wildlife are often adversely affected.

Gulf Coast Tick

The Gulf Coast tick, Amblyomma maculatum Koch, is an important pest in the states bordering the Gulf of Mexico. This tick is a native of North, Central, and South America. In the United States its distribution is restricted to the Gulf Coast and Coastal Atlantic regions from South Carolina to Texas (Bishopp and Trembley, 1945). Bishopp and Hixson (1936) state that this tick is seldom found in significant numbers more than 100 miles inland and that its distribution suggests that rather high rainfall, humidity, and temperature are necessary for its existence.

The Gulf Coast tick attaches itself largely on the inner surface of the external ear of various animals. Its concentration in these places causes irritation, often resulting in scabbing and cracking of the skin. This condition is distinctly detrimental to livestock. The principal injury is indirect. The ear lesions attract the primary screwworm fly which lays its eggs on them. The mass of ticks in the ear causes it to swell and become stiff. This prevents the host from flicking the ear and thus permits blowflies to oviposit undisturbed. The resulting larvae burrow into the ear tissues, and the blood and serum discharged attract other flies. If treatment is neglected the skull may be laid bare and the animal may die. Even when the larvae are killed rather promptly, the cartilage of the ear is often destroyed so that the ear droops producing the characteristic "gotch ear" so frequently seen, especially among mules. This type of injury has given rise to the common name "gotch tick" in some localities (Bishopp and Hixson, 1936). The hosts of the adults are similar to those listed for the adults of _A_. _americanum_ with sheep, horses, mules, and cattle most often infested. The larvae and nymphs, as reported by Bishopp and Trembley (1945), engorge mainly on ground-inhabiting birds, although they are also found on small animals. They listed 21 species of birds as being parasitized, mainly by the immature stages.

Amblyomma americanum and A. maculatum are both 3-host ticks. That is, the larvae and nymphs drop off the host to moult; thus the larvae, nymphs, and adults may, and usually do, engorge on 3 different hosts. Their life cycles in nature usually require 1 to 2 years. There are 4 stages: the egg, the larva or seed tick, the nymph, and the adult. Each instar requires 1 to 2 weeks for feeding while attached on different types and sizes of hosts. Under laboratory conditions, Loomis (1961) obtained a life cycle of A. americanum in a minimum of 71 days. Garnett and Sacktor (1947) obtained a life cycle of 3 months for this species at 30°C and between 85 to 100 per cent relative humidity.

Eggs

The eggs of A. americanum are deposited in a sticky mass in front of the engorged female. The color of eggs ranges from dark, reddish brown to a light tan. The number laid by an engorged female is about 4,000.

The eggs of A. maculatum are ovoid and dark brown. The average number of eggs laid is about 8,000 although some engorged females may deposit as many as 18,000 eggs (Bishopp and Hixson, 1936). Generally 70 to 90 per cent of the eggs of both species hatch and the mean length of the incubation period of the egg masses is 25 days. The weight of a single egg is about 0.054 mg.

Larvae

At room temperature (21°C) larvae can survive for about 10 to 12 weeks. A 1- to 3-week interval is required to allow almost 100 per cent of the larvae to complete cuticle hardening. In this manner, a large proportion attach to their initial host and larval mortality is reduced to a minimum. During the cuticle hardening period, readiness to feed is indicated by the presence of white excretory material deposited by the larvae on the walls of the vials (Loomis, 1961). Engorgement usually requires 4 to 7 days.

There are no visible signs of engorgement of the larvae during most of the attachment period. Noticeable enlargement occurs a few hours before the larvae are fully repleted and are ready to detach from the host. The fully engorged larvae weigh about 1 mg which is approximately a 24-fold increase over weight at hatching. Moulting to the nymphal stage occurs 18 to 26 days after detachment.

Nymphs

One to 2 weeks are required before the nymphs are ready to attach to a new host. Attachment to the host occurs within a few hours after initial contact with the host. As with the larvae, engorgement is not apparent during the first few days of attachment, but becomes noticeable approximately 2 days before the fully engorged nymphs detach. Engorgement usually requires 4 to 7 days. A moulting period of 14 to 28 days is required.

Adults

After emerging as adults another quiescent period of about 2 weeks is required before feeding. Engorged Gulf Coast females are larger than any other North American tick except the gopher tortoise tick, Amblyomma tuberculatum Marx. The engorged female may attain a weight of 1 gram and measure almost 19 mm. The average weight is 460 mg.

The time of initiation of feeding by adult ticks varies. Some ticks attach immediately to the host while others do not attach for several days. Mating takes place on the host. Apparently, females do not generally engorge to completion in the absence of males. Both males and females feed before copulation.

Oviposition

The female may oviposit almost immediately after mating, or wait several weeks before initiating oviposition. There appears to be no correlation between the time of engorgement and the preoviposition period nor is there any apparent relationship between the number of eggs laid and the preoviposition period. The mean preoviposition period is 6.5 days (Sacktor et al., 1948). The number of eggs that a female tick can lay is proportional to the engorged size of the tick (Gladney and Drummond, 1970); the larger the size of the tick, the more eggs laid.

The Feeding Mechanism of Ticks

The elongate mouthparts possessed by the members of the genus Amblyomma are important in their role as disease vectors and pests. The ticks have a well-developed capitulum which consists of a heavily sclerotized rectangular collar, the basis capituli which bears on its antero-lateral margins a pair of 4 segmented palps and contains within it a pair of protrusible shafts, the chelicerae or cutting organs. The chelicerae are believed by some authors (Arthur, 1946) to be homologous with the mandibles of blood-sucking organs. Snodgrass (1948), however, considers them to represent the corresponding second antennae of Crustacea or their homologues, the vestigial premandibular appendages of insects. The ventral portion of the basis extends anteriorly beneath the chelicerae as the spoon-shaped hypostome; dorsally it is prolonged to partially ensheath the chelicerae. The longitudinal space between the chelicerae and hypostome forms the buccal canal and buccal cavity. Posteriorly the large basal portion of the basis is neck-shaped and fits within an opening in the body of the tick known as the camerostome. A reflexed articulating membrane connects the two margins permitting a limited dorsoventral movement of the capitulum and a flow of blood from the body cavity to the extremities of each of the five mouthparts (Gregson, 1960). Snodgrass (1948) in reviewing the homology of arachnid feeding organs, considers the coxae of the palps as forming the sides of the basis capituli and uniting ventrally and distally to produce the hypostome.

Two actions appear to occur within the capitulum when a tick is feeding. The first movement accompanies the ejection of saliva into the wound and is caused by the action of the anterior subpharyngeal muscles. The second movement within the capitulum of the feeding tick is that of the pharynx and occurs when the tick is sucking (Gregson, 1960).

The Feeding Mechanism in Relation to the Host

The factors, both inherent and environmental, which motivate ticks to obtain their blood meals constitute an important aspect of their ecology. The succession of actions leading to normal attachment to a host may be said to begin when the tick comes into a questing state. According to Lees (1948) the unfed sheep tick, Ixodes ricinus (L.), when at rest adopts either a "questing" attitude with the forelegs extended or an attitude of repose with the forelegs folded. While walking, the hungry tick waves the forelegs in the manner of antennae.

Factors Influencing Attachment to the Host

Attachment to the host for feeding purposes by ticks is determined by the interaction of groups of some organs, viz. Haller's organ, temperature and tactile bristles on the legs, palpal organs, and chemoreceptors on the palpal segments (Lees, 1948; Arthur, 1953).

Attachment is greatly influenced by the age (therefore, the state of hunger) of ticks. The longer the period of starvation, the faster they proceed to attach (Lees, 1948). Starvation resulting from the absence of an appropriate host stimulates the ticks (immatures and adults) to take larger blood meals (in comparison to unstarved ticks) eventually resulting in larger females producing greater numbers of eggs (Amin, 1969). Some ticks never attach, regardless of their age. There is little difference in attachment rate between the sexes. Attachment rates increased from 1 to 3% at 1 day after moulting to more than 70% at 8-9 days after moulting (Gladney et al., 1970).

The attraction of ticks to membranes and hosts is also conditioned by temperature. Lees (1948) showed that below 20°C unfed females of the sheep tick are indifferent to the smell of sheep's wool alone. The attractiveness of sheep's wool for attachment is increased by raising the temperature from 20° to 37°C. Temperatures higher than 42°C have a repellent effect. Odor and temperature acting together stimulate greater attachment than either stimulus alone. Thus hungry adults, which are repelled by temperature alone, are attracted when both are presented together. Obviously, body temperature is an inducement for attachment, for when the body temperature of the host is lowered, as in death, the ticks do not attach or if attached, detach and leave the host.

Hindle and Merriman (1912) found that Argas persicus (Oken) with amputated forelegs (hence the removal of the odor-perceiving organ) attach themselves to artificial membranes made from rat diaphragm when the latter were placed in 0.8% sodium chloride or gelatine solutions at a temperature of 42°C. Under similar conditions normal ticks failed to attach. These results are indicative of the role of odor as a prerequisite for attachment to hosts (Arthur, 1965).

All ticks require a high relative humidity (Hooker et al., 1912; Bishopp and Hixson, 1936). Sacktor et al. (1948) showed that in the laboratory a relative humidity of from 85 to 100% is most favorable for lone star ticks. The response to humidity is greatly influenced by the physiological state. When the water balance is normal unfed ticks avoid higher humidities but come to rest with equal readiness in moist or dry air (Lees, 1948).

Unfed ticks at rest on a glass rod respond to a sudden fall in light intensity by questing. Engorged ticks are strongly photonegative; newly moulted ticks avoid direct illumination, but become indifferent with age (Lees, 1948). Granett and Sacktor (1947) obtained a photopositive reaction from lone star ticks; both nymphs and adults exhibited an attraction to light. In their work with the American dog tick, Dermacentor variabilis (Say), Atwood and Sonenshine (1967) found a relationship between solar radiation levels and daily questing activity of adults. Continuous adult activity occurred during the periods when the daylight exceeded 13 hours and an average solar radiation level of about 300 langleys was received in the habitat. Lancaster (1955) suggested that the decreasing day length was responsible for the cessation of feeding activity. Work by Gladney and Wright (1971) showed that ticks exposed to longer constant photophases had progressively higher rates of attachment. Similarly the rate of attachment of ticks exposed to continuously increasing light was significantly greater than that of ticks exposed to continuously decreasing light.

The presence of other ticks attached to hosts stimulates the tick to attach. Gladney (1970) reported that attached male Gulf Coast ticks increased the rate of attachment of females, whereas females alone did not attach readily.

Investigations of factors which can induce feeding in Ornithodorus tholozani (Laboulbène and Megnin) by Galun and Kindler (1968) have shown that this tick imbides saliva containing reduced gluthathione and glucose as readily as whole blood. The effect of these two compounds was synergistic when tested at concentrations found in blood. Glucose could not be replaced by fructose, galactose, arabinose, or sucrose. In addition, carbon dioxide acts as an attractant and activator for certain ticks (Wilson et al., 1972).

After prolonged starvation, marked feeding response could be elicited by isotonic saline containing glucose and a specific L-amino acid. Solutions containing ATP or DNPH in place of gluthathione also induced maximal feeding response.

Host nutrition and behavior also play dominant roles in the attachment of ticks. The nature of the resistance of cattle fed a high protein and fat diet to the cattle tick, Boophilus annulatus (Say), appears to be physiological rather than behavioral (Gladney et al., 1973). With A. maculatum the natural tendency of ungulates to swing the ear to and fro when stimulated by movement on the hair in the ear, is a dominant factor inducing the adult to attach. The ear reflex causes the tick to cease crawling and cling to the hair. Repeated attempts to disengage the tick causes it to insure its position by going deeper among the hairs. In this position, the tendency to crawl ceases and attachment follows (Hixson, 1940).

The Process of Attaching

Attachment begins by the application of the tip of the hypostome and chelicerae to the skin of the host. This action is immediately followed by alternate outward lacerating movements of the digits. Attachment is accomplished by the secretion of a "cement" substance in which the mouthparts are embedded and which adheres firmly to the host. This cement functions in promoting firm and early anchorage during the long attachment of Ixodid ticks to a host. The nature and origin of the cement substance in Haemaphysalis spinigera Neumann is discussed by Chinery (1973). In females, it consists of external cement forming a cone, with a substantial lateral extension into the more superficial skin layers, and internal cement. The internal cement, which is a continuation of the cement cone and which extends deeper into the skin, consists of a tapering tube surrounding the mouthparts, other than the palps which are flexed laterally outside the cone. The hypostomal teeth lock with corresponding impressions on the inner surface of the tube, while the outer surface of this tube takes the form of strand-like protrusions of cement extending between fibrils of the deep dermal tissue. The overall mass of cement in the host's skin may be as much as 0.042 mm^3. Males show similar features but the wing-like extension of the cone and the strands from the tube in the skin are much reduced. Histochemical studies indicate that the cement is mainly protein and is derived from the salivary glands.

The start of the secretion of cement and initial cutting of the host tissue by the chelicerae occur simultaneously. Final rapid engorgement of each stage is preceded by a secondary secretion of cement into a fluid-filled cavity which forms directly beneath the mouthparts and provides additional support (Moorhouse and Tatchell, 1966).

The Process of Feeding

Attachment is followed by an intermittent probing and cutting of underlying tissues by the digits. The alternate action of salivation and sucking varies in intensity and time depending on the tick's rate of feeding. The periods of sucking and secreting may also be interrupted by periods of inactivity during which there is no apparent movement of blood or saliva (Gregson, 1960).

The salivation into host tissue at frequent intervals substantiates the theory that tick paralysis is caused by some action of the tick's saliva on the host (Gregson, 1953). The protein components of the tick extracts are largely responsible for the development of skin hypersensitivity (Riek, 1958).

The Process of Detaching

The final response to attachment occurs either when the tick reaches repletion or when it is accidentally detached from its host. Prior to this time the tick is so firmly attached to the skin by its cement-like secretion that only a strong pull will dislodge it. Even then, the cone-shaped papilla is usually pulled away with the mouthparts, and normal separation is so difficult that the capitulum can be torn off by the pull (Gregson, 1960). Relaxation of the dilated mouthparts upon completion of engorgement would loosen the interlock of the hypostomal teeth with the corresponding indentations in the cement tube and facilitate detachment of the replete tick (Chinery, 1973).

Sense Organs of Ticks

It has been known for some time that stimuli affecting the behavior of ticks are perceived by sense organs in the surfaces of the body and on various appendages. Among these sense organs are (1) numerous cylindrical, slightly curved setae in the body integument and on the legs; (2) the special sensory structure called Haller's organ on tarsus 1; (3) sensilla; (4) the porose areas; and (5) the fovae (Douglas, 1943; Lees, 1948; Arthur, 1953, 1961, and 1962; and Woolley, 1972).

Setae of the Body and Legs

Little is known of the specific functions of the hairs on the idiosoma of ticks, although these hairs generally resemble the sensory setae on the palpi and certain setae on the legs. Each of the short setae found on both the scutum and alloscutum in hard ticks is known to rest in a socket over the terminal opening of a canal that penetrates the cuticle (Arthur, 1961). The pattern of their distribution on the surface, particularly on the scutum, varies with the species, but the functions appear to be similar and to range from tactile to chemosensory responses (Woolley, 1972).

The distal three segments of tick legs (tibia, metatarsus, and tarsus) exhibit long, curved setae on the ventral surface. Both tactile and thermal stimuli are received by these structures. The setae are curved, with their pointed apices directed toward the claws. Temperature sensitive setae are shorter than the longer, tactile setae. The short, straight setae of all legs are also hollow and are assumed to be the principal temperature receptors (Arthur, 1961; Wooley, 1972). As in the case of the integumental setae of the scutum and alloscutum, both types of leg setae are thought to rest over canals that penetrate through the cuticle (Arthur, 1961).

Haller's Organ

Haller's organ is located on the dorsal surface of the tarsi of the first pair of legs of both soft ticks and hard ticks. It consists of a trough and a capsule. The external trough usually exhibits six sensory setae with four types distinguishable. The posterior capsule of Haller's organ is somewhat oval in shape with curved sides and a thin transparent roof (Arthur, 1961).

Ticks detect humidity changes and find their hosts by means of Haller's organs. Illustrations and descriptions in the literature depict ticks with their front legs extended forward in a stationary position or waved in a manner similar to the movement of insect antennae. The reactions of these ticks to changes in atmospheric humidity (Arthur, 1961) indicate that the sensilla of Haller's organs function as hygrometers.

It has been demonstrated also that the capsule of Haller's organ is an odor-perceiving structure, but in this sense it also functions in conjunction with the sensilla at the apex of the palpal tarsus. Experiments have shown that when ticks are deprived of the use of Haller's organ, but the palpi remain functional, the ticks cannot find a host; but if placed on the host may begin to feed. On the other hand, if the palpi and the mouthparts are prevented from functioning, but the Haller's organ is intact, the ticks are able to find the host, but they cannot feed and die if left in this condition. Both the sensilla in Haller's organ and those on the palpal tarsal segment are considered chemoreceptors (Woolley, 1972).

In _A_. _americanum_ the porosity of the large, truncate sensillum in Haller's organ is pronounced. The lip-like structures adjacent to the sensilla in Haller's organ are of unknown function and seem to be peculiar to this species (Woolley, 1972).

Sensilla of Palpal Segment 4

There are 2 types of sensilla on palpal segment 4 of _A_. _americanum_ and _A_. _maculatum_; a cluster of ten rather heavy blunt hairs on the ventral hemisphere, and five more slender filiform sensilla.

The palpal organ, which is situated at the tip of the palp on the ventral surface, functions with the Haller's organ to perceive odors.

Porose Areas

Two perforated depressions called porose areas are found in the dorsal surface of the basis capitulum of females of species of hard tick ticks. Each porose area consists of a concavity containing pores and a group of ducts innervated by the dorsal ganglionic portion of the brain (Arthur, 1961). These areas are described by Douglas (1943). Their exact function is not known, but it is assumed that they are chemoreceptors which augment the function of the hairs on the palpal tarsi and other parts of the body.

The Fovae

The fovae are morphologically similar to the porose areas and have been regarded as sense organs by Douglas (1943). In Dermacentor, these organs are found in both sexes. They are situated directly over the heart. Their function is unknown.

Mating Behavior and Reproduction

The mating habits of most Ixodid tick species are rather poorly understood. Especially important is the knowledge of the number of times the male of any tick species will mate, a prerequisite to any study dealing with control of a tick species by the sterile-male technique (Drummond et al., 1966).

The mating behavior of male ticks has particular importance, because the male is far more active than the female in seeking a mate and in initiating copulation. Sacktor et al. (1948) stated that once the female of the lone star tick attaches, she rarely moves, but the male frequently moves over the host seeking females. Hooker et al. (1972)

observed that only a short time is required for fertilization of female lone star ticks, since males remain in copulation for only a few hours then pass to other females or return to the same female. These workers reported also that males were frequently found mating with fully engorged females.

Hixson (1940) stated that the male Gulf Coast tick must remain attached 6 or 7 days before it can be induced to copulate. The male remains attached in one place throughout the precopulation period. Sexually mature males are stimulated to activity by other ticks crawling over them. When stimulated, sexually mature males detach and seek attached or free, fertile or infertile females with which they copulate.

Copulation in ticks involves the male crawling under the female so that their venters are adjacent. There is an initial stimulatory period in which various parts of the male capitulum are associated with the female genital aperture (Oliver, 1974).

Male ticks are capable of inseminating many females. Hixson (1940) stated that one male Gulf Coast tick inseminated 6 females within 5 days. An individual male lone star tick inseminated 27-37 females (probably copulated with 29-40 females) during periods of 68-127 days on hosts (Gladney and Drummond, 1970). The mating behavior in lone star and Gulf Coast ticks is elicited by a sex pheromone secreted by the female to attract the male for mating (Berger et al., 1971).

Under natural conditions most hard ticks, except many species of the genus Ixodes, mate exclusively on hosts (Arthur, 1962). However, during the course of studies on the mating habits of lone star ticks, Gladney and Drummond (1970, 1971) observed that prefed males would copulate with and transfer spermatophores to unfed virgin females off the hosts (in vials).

Oviposition in ticks is usually dependent upon feeding and mating, although parthenogenesis (Oliver, 1974) and autogeny (Feldman-Muhsam, 1973) occur in some species. Parthenogentic individuals require feeding and autogenous ones copulate prior to oviposition. No reports of any ticks that are both parthenogenetic and autogenous were found. Parthenogenesis exists in the Argasidae and Ixodidae, but autogeny is reported only in certain of the Argasidae.

Factors Affecting Seasonal Abundance and Distribution of Ticks

The lone star tick is much more abundant in certain habitats than others. It is felt that various environmental factors play an important role in the distribution of this pest; attempts have been made to correlate the abundance and distribution of the lone star tick with ecological habitat-type and environmental conditions (Bishopp and Trembley, 1945; Brennan, 1945; Lancaster, 1957; Sonenshine et al., 1966; Hoch et al., 1971). Bishopp and Trembley (1945) reported that heavier infestations of lone star ticks were usually found in wooded areas as opposed to areas with less dense vegetation. Lancaster (1957) believed that unimproved pastureland with dense brushy vegetation supported higher numbers of lone star ticks than improved pastures, and Semtner et al. (1971) found a close correlation between the number of ticks present and the density of the vegetation.

Types of vegetation and more specifically, the environmental conditions created by these different habitats have been of principal concern to several lone star tick researchers (Lancaster, 1957; Sonenshine et al., 1966; Semtner et al., 1971). Wilson et al. (1972) and Semtner et al. (1971) presented conclusive proof that the numbers of lone star ticks found within a general area depend on the physical makeup of existing vegetation.

In studies by Semtner et al. (1971) the longevity of caged adults of the lone star tick was found to be shortest in a meadow habitat and longest in a bottomland oak-hickory habitat. Data collected indicate that mid-summer temperatures influence the behavior of adult ticks in all habitat types. As a behavioral response to rising temperatures adults migrate down vegetation to the soil.

The vertical migration of I. ricinus has been correlated with body water content (Lees and Milne, 1951). When there was little or no moisture deficit in these ticks, they would move up on the vegetation, and when the moisture deficit reached a critical point, they would come down into the moist floor and replenish their water losses. Lone star ticks, like I. ricinus (Lees, 1948), are able to reabsorb water from environments with humidities above the critical equilibrium humidity of the ticks (Sauer and Hair, 1971).

The Gulf Coast tick, as stated earlier, is restricted in its distribution, but increasing numbers have become established in areas outside their normal range. The transportation of infested hosts to those areas is probably responsible for their presence there (Semtner and Hair, 1973).

III. METHODS AND MATERIALS

Tick Rearing Procedures

Colonies of lone star and Gulf Coast ticks were established in the laboratory from engorged females collected from dogs and deer. The procedures followed in rearing the 2 species were quite similar. The feeding stages of the ticks were confined on rabbits in cages constructed from the screw-top and neck portion of 1 quart polyethylene Mason jars (Gregson, 1966). The jar tops were shaped to fit the contour of the rabbit's body. Contact cement and elastic tape were used to secure the cages to the rabbit. Two cages were placed on each rabbit providing a circular feeding area of approximately 6 cm in diameter. The jar opening was covered with fine mesh screen held in place by conventional Mason rings to permit ventilation and prevent the accumulation of moisture. Rabbits were used only once due to the long period of time required for the feeding injuries to heal and the apparent buildup of an immune reaction to ticks introduced later.

The larvae that hatched from each egg mass fed on the exposed skin area inside each cage. The engorged larvae were collected from the cages and held in glass vials until they moulted and were ready to feed. The emerged nymphs were also held in glass vials.

The number of nymphs fed in each cage averaged about 1,000. The collection and holding procedures were the same as for the larvae.

The emerging adults were separated according to sex and isolated in separate containers to avoid contact with members of the opposite sex. The adults were aged for several weeks before being used.

Eggs needed for maintaining the colonies were obtained by periodically feeding males and females together and collecting the engorged females that dropped from the host. Each engorged female was placed in a small cardboard pill box (1 inch diameter), and the box was covered with Saran Wrap to permit easy inspection. The eggs were laid and hatched, and the larvae were held in these boxes until ready to feed.

When not on a host, all stages of the tick were held in a growth chamber programmed to maintain an 11-hour dark phase at 23 ± 1°C and 80 ± 15% relative humidity and a 13-hour light phase at 27 ± 1°C and 80 ± 15% relative humidity. The lights in the animal room where the ticks were held while feeding were controlled on a similar cycle. The temperature of this room was maintained at 24 ± 3°C but no attempt was made to control the humidity.

Bioassay and General Procedures

The host animals used in all experiments were domestic white rabbits weighing 5 to 7 pounds. The 2,6-dichlorophenol solutions used in the bioassay were prepared by dissolving synthetic crystalline 2,6-dichlorophenol in benzene or acetone. The concentration of 2,6-dichlorophenol used in all tests was 5 μg/μl. The solution was applied to the skin of the host with a 10 μl capacity syringe.

The ticks were confined on the host animals with two types of cages. The first of these was the same as those used in the tick rearing procedures described above. Two cages were attached to the sides of each rabbit between the fore and hind legs. Before attachment of cages, the back and sides of the rabbit were clipped with an electric pet clipper so that the ticks could contact the skin readily and tick movements and attachment behavior could be observed. In clipping the animal, care was taken to avoid cutting the skin because wounds or oozing blood may have affected the attachment and feeding of the ticks. The cement used to attach the cages to the host was allowed to dry thoroughly before ticks were placed inside the cages because the solvent in the glue might act as a fumigant (Sacktor et al., 1948). In these cages the ticks were confined in a small restricted area, so attachment and feeding behavior could be observed easily.

A cage that would enclose a larger area of the host's body surface was needed to study migratory movement of ticks that might be promoted by the chemical. Such cages were constructed from a 1/2 × 4 × 12 inch rectangular piece of flexible polyethylene foam with a 2.5 × 10 inch opening in the center. The opening was covered on the outside with copper screen, and the cage was attached over the back of the rabbit with contact cement and elastic tape. The area of study in this cage extended over the back and down both sides of the rabbit.

Comparison of the Content of 2,6-Dichlorophenol in the Sexes of the Ticks

Samples of both Gulf Coast and lone star ticks consisting of adults differing in age and sex were analyzed to determine their 2,6-dichlorophenol content. A large group of nymphs of each species was fed, collected as they dropped from the host, and observed periodically for signs of moulting. As adults began to emerge, they were removed daily, sexed, and divided into 6 separate groups. The adults were held for various time intervals then frozen. The process was continued until 2 samples of 10 adults each of each sex of each species which had been held 0, 2, 4, 8, 16, and 32 days before they were frozen had been assembled.

Extracts of the various samples of ticks were made by adding 1 ml of benzene to 4 dram screw cap vials in which the ticks had been stored and placing the vials with their contents in a sonic cleaning bath for 1 hour. The 2,6-dichlorophenol content of the extracts was determined by gas chromatography using a 2 mm I.D. × 3 m column of 3% Hieff 8 BP on 100/120 mesh Gas Chrom Q and an electron capture detector. The flow rate of the carrier gas was 26 cc/min. Standard solutions containing known quantities of the phenol were used to calibrate the detector's response and for reference in determining the retention time of the phenol in the samples. Calculations (ppm basis) were based on the weight of the ticks.

Comparison of the Attractiveness of 2,6-Dichlorophenol to the Sexes of the Ticks

Lone star males show a much more obvious reaction to the presence of 2,6-dichlorophenol than females. The female possesses much greater quantities of the chemical. The conclusion that the chemical serves as a sex pheromone or sex attractant in this species is logical.

The situation has been shown to be somewhat different in Gulf Coast ticks. There has been disagreement among investigators with regard to which sex is attracted to the other in mating. Chemical analyses have shown that both sexes of this species contain a moderate amount of 2,6-dichlorophenol with females containing slightly more than males. This difference in the distribution of the chemical between the sexes of the 2 species of ticks might explain the apparent difference in their mating behavior.

In most tick species the male seeks the female. Only in the genus Amblyomma have deviations from this norm been shown. In Africa Lounsbury (1899) reported that the male bont tick, Amblyomma habraeum Koch, probably attracts females (Gladney, 1971; Oliver, 1974). Gladney (1971) believes that the males of A. maculatum attract females. The purpose of this research, therefore, was to determine to what extent the individual sexes of these two species were attracted by the phenol.

The presence of ticks already attached on a host has been reported to have an effect on the assembly and attachment of other ticks at this site (Gladney, 1970). Therefore, the test chemical was applied in the vicinity of feeding ticks and observations were made to determine if ticks attached at another site were attracted to the treated area.

The tests were run in 2 parts; once in the spring (April-May, 1973) and again in the fall (Sept.-Nov., 1973). Four series of tests were run; 2 series with Gulf Coast and 2 with lone star ticks. Twelve rabbits were used in each series of tests, with the same procedures being used for both. Two groups of unfed adult ticks of the same sex were placed in separate small polyethylene cages on both sides of the rabbit and allowed to attach. Then the cages were removed and replaced by a larger, single flexible polyethylene foam cage. The ticks on both sides had complete freedom to move to either side. Fifty μg of 2,6-dichlorophenol dissolved in 10 μl of acetone was applied to the skin of the host animal near the site of attachment of one group of ticks. The chemical applications were repeated every 2 days for one week. The migration of the ticks was checked every day for 7 days. After 7 days, the cage was removed and the number of ticks distributed to either side of the backline of the host animal was determined. The test was replicated 3 times with each sex. Three controls of each sex which received no chemical were also included in each series.

IV. RESULTS AND DISCUSSION

Berger (1971) obtained indications that only female lone star ticks contained appreciable amounts of 2,6-dichlorophenol. Analyses of male and female adults of various ages revealed that the phenol did not occur in measurable amounts in newly emerged adults. It began to show up in fair amounts in females by the 2nd day after emergence and continued to increase for several days. It appeared to reach a plateau of about 6 ppm in females at 2 to 3 weeks (Figure 1). The level in males did not exceed 0.1 ppm at any time. Its presence in males does not appear to be an artifact, however. Males carefully separated from females at the time of emergence and held for several weeks before being analyzed all contained this small amount of phenol. There appeared to be a decrease in phenol content after about 4 weeks (Figures 1 and 2). Possible explanations for the decrease in phenol content may be that it is excreted, metabolized, broken down (degraded), or is lost through evaporation. The reasons for the decline in phenol content remain unclear and need further investigation.

Analyses of unfed Gulf Coast ticks revealed somewhat different results. Both sexes contain about equal amounts of the phenol. Sixteen day-old males and females contained 2.4 and 1.8 ppm of 2,6-dichlorophenol, respectively (Figure 2). The amounts obtained in this research were about the same as those reported in samples of Gulf Coast ticks tested by Berger (personal communication). Four day-old males contained 1.1 ppm and similar aged females contained 1.4 ppm of the phenol. This difference in the distribution of the phenol between the sexes might explain the apparent difference in the mating behavior of these tick species. It seems quite clear from this study that male lone star ticks seek the females for mating. It would seem logical that this is the case if the phenol acts as an attractant since, the females are the only sex that produces it in significant quantities. By this reasoning, the distribution of 2,6-dichlorophenol in Gulf Coast ticks should result in a mutual attraction between sexes but, this was not verified by this research.

There appeared to be no distinct effect of 2,6-dichlorophenol on movement of female ticks of either species on the host (Tables 1 through 4). The data indicated that movement by the females was random. An apparent negative response of the females to the compound was observed in some tests (Table 1). This negative response could have been a result of agitation caused by a high concentration of the chemical that was added or to a response to the attachment of other ticks at another site.

There was an indication that 2,6-dichlorophenol affected the movement and attachment of the males. By comparing the data obtained on the response of males and females, it appears that the phenol caused the males to move in greater numbers toward the site of application of the compound (Tables 5 through 8). The males exhibited more positive responses to the chemical than negative responses. The males on the treated animals were more agitated and tended to aggregate in clusters more than the males on the controls.

Attached males of both species detached and migrated toward the site of application of the chemical on rabbits treated on one side with

2,6-dichlorophenol. Seven days after the compound was first applied approximately 80% of the ticks on the hosts were on the treated sides (Tables 5 through 8). The movement of males on the control rabbits was insignificant; the slight movement was probably a result of chance, because male ticks usually move over the host if they remain on the host for long periods (Sacktor et al., 1948).

Behavioral responses similar to those reported by Berger et al. (1970) were also observed. The males were noticeably agitated each time the compound was applied. These responses were not immediately evident in the female ticks.

Both sexes of lone star ticks attached more quickly than Gulf Coast ticks; most of the lone star ticks having attached in an average of 7 days as compared to an average of 12 days for Gulf Coast ticks (Tables 1 through 8) otherwise, there was little difference in the movements of the 2 species of ticks. As expected, the males on treated hosts tended to move toward the chemical. Males on untreated hosts and females on treated as well as untreated hosts showed little or no signs of directed orientation.

Since both sexes of Gulf Coast ticks contain detectable amounts of 2,6-dichlorophenol, one might expect a mutual attraction of both sexes for one another. Only the results in Table 1 reveal any evidence of Gulf Coast females tending to migrate toward the compound. Despite a negative response of the ticks on Rabbit A to the chemical, 47% of the ticks were on the treated sides of rabbits A, B, and C (Table 1) as compared to 49% on the untreated sides at the termination of the tests. The rest of the ticks were either unattached or on the middle of the back of the host. The results from the second series of tests run on Gulf Coast females indicated an average of 35% of the ticks on the treated sides and 42% on the untreated sides (Table 2) at the end of the experiment.

The data on the movement of ticks in response to 2,6-dichlorophenol were statistically analyzed by an analysis of variance. This analysis revealed that the males were definitely attracted to the phenol. The movement by the males was significant at the 5% level. The results were somewhat different in the case of the females. The influence of the chemical on female movement was non-significant. Also there was no significant difference (5% level) between attachment and distribution of ticks on the rabbits prior to initiation of the chemical treatments.

The experiments conducted by Gladney (1971), in which he reported that unfed Gulf Coast females were attracted to males, were designed to test only the mate seeking of females. He did not conduct the opposite experiment to test attraction of males to females. The data presented here shows evidence that males are attracted to synthetic 2,6-dichlorophenol. The evidence in the case of the females is marginal.

V. SUMMARY

The 2,6-dichlorophenol content of the sexes of unfed Gulf Coast ticks, Amblyomma maculatum Koch, and lone star ticks, Amblyomma americanum (Linnaeus), of various ages was determined by gas chromatography. Significant amounts of the compound were found in female lone star ticks and reached a plateau of 6-7 ppm in 2-3 weeks. Only traces of the phenol were detected in males (Figure 1). Both sexes of Gulf Coast ticks contained appreciable amounts of the compound; 2-3 week-old males and females contained 2.4 and 1.8 ppm, respectively (Figure 2).

Tests were also conducted to determine the attractiveness of the compound to the sexes of the 2 species of ticks. The ability of the chemical to cause the aggregation of individuals in a treated portion of a confined area extending over the back and down the sides of white rabbits was assessed.

Data revealed that both species of males were attracted to the chemical. There was an indication that females were also attracted to the chemical but the evidence was very marginal.

Table 1. Distribution of Previously Attached Female Ticks (Gulf Coast) on Rabbits 7 Days After Initial Application* of 2,6-Dichlorophenol (50 adults/rabbit). Series 1.

Replicate No.	No. of Ticks Attached	Side of Rabbit	Dosage	Ticks/Side 7 Days Later	% of Ticks Attached 7 Days Later
Rabbit A	31	6 R 25 L	50 µg	1 30	3
Rabbit B	44	24 R 20 L	50 µg	15 26	63
Rabbit C	41	21 R 20 L	50 µg	10 28	74
Rabbit D	41	18 R 23 L	Control	18 22	45
Rabbit E	44	20 R 24 L	Control	20 21	51
Rabbit F	44	21 R 23 L	Control	22 22	50
					se 12.66

No significant difference between treatment and control at 5% level.

Replicates	No. of Ticks Attached	No. Moved on Treated Rabbits	No. Moved on Control Rabbits
6	245	19	1

% of ticks on treated sides of treated rabbits - 47
% of ticks on corresponding sides of control rabbits - 49

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[*] Subsequent applications made at 2-day intervals.

Table 2. Distribution of Previously Attached Female Ticks (Gulf Coast) on Rabbits 7 Days After Initial Application* of 2,6-Dichlorophenol (50 adults/rabbit). Series 2.

Replicate No.	No. of Ticks Attached	Side of Rabbit	Dosage	Ticks/Side 7 Days Later	% of Ticks Attached 7 Days Later
Rabbit A	42	24 R 18 L	50 μg	26 16	38
Rabbit B	41	23 R 18 L	50 μg	26 15	37
Rabbit C	29	21 R 8 L	50 μg	20 9	31
Rabbit D	37	19 R 16 L	Control	19 16	42
Rabbit E	24	15 R 9 L	Control	13 9	37
Rabbit F	44	23 R 21 L	Control	23 21	48
					se 2.28

No significant difference between treatment and control at 5% level.

Replicates	No. of Ticks Attached	No. Moved on Treated Rabbits	No. Moved on Control Rabbits
6	217	6	0

% of ticks on treated sides of treated rabbits - 35
% of ticks on corresponding sides of control rabbits - 42

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*Subsequent applications made at 2-day intervals.

Table 3. Distribution of Previously Attached Female Ticks (Lone Star) on Rabbits 7 Days After Initial Application* of 2,6-Dichlorophenol (50 adults/rabbit). Series 1.

Replicate No.	No. of Ticks Attached	Side of Rabbit	Dosage	Ticks/Side 7 Days Later	% of Ticks Attached 7 Days Later
Rabbit A	45	25 R 20 L	50 μg	25 20	56
Rabbit B	50	25 R 25 L	50 μg	29 21	58
Rabbit C	50	25 R 25 L	50 μg	27 23	54
Rabbit D	45	25 R 20 L	Control	24 20	53
Rabbit E	46	24 R 22 L	Control	24 22	52
Rabbit F	49	25 R 24 L	Control	25 24	51
					se 0.742

Significant difference between treatment and control at 5% level.

Replicates	No. of Ticks Attached	No. Moved on Treated Rabbits	No. Moved on Control Rabbits
6	285	5	1

% of ticks on treated sides of treated rabbits - 56
% of ticks on corresponding sides of control rabbits - 52

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*Subsequent applications made at 2-day intervals.

Table 4. Distribution of Previously Attached Female Ticks (Lone Star) on Rabbits 7 Days After Initial Application* of 2,6-Dichlorophenol (50 adults/rabbit). Series 2.

Replicate No.	No. of Ticks Attached	Side of Rabbit	Dosage	Ticks/Side 7 Days Later	% of Ticks Attached 7 Days Later
Rabbit A	50	24 R 26 L	50 µg	24 26	52
Rabbit B	40	24 R 16 L	50 µg	25 15	63
Rabbit C	44	20 R 24 L	50 µg	20 22	50
Rabbit D	38	25 R 13 L	Control	25 13	34
Rabbit E	45	23 R 22 L	Control	23 20	51
Rabbit F	48	23 R 25 L	Control	23 25	52
					se 4.14

No significant difference between treatment and control at 5% level.

Replicates	No. of Ticks Attached	No. Moved on Treated Rabbits	No. Moved on Control Rabbits
6	265	1	0

% of ticks on treated sides of treated rabbits - 55
% of ticks on corresponding sides of control rabbits - 46

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*Subsequent applications made at 2-day intervals.

Table 5. Distribution of Previously Attached Male Ticks (Gulf Coast) on Rabbits 7 Days After Initial Application* of 2,6-Dichlorophenol (50 adults/rabbit). Series 1.

Replicate No.	No. of Ticks Attached	Side of Rabbit	Dosage	Ticks/Side 7 Days Later	% of Ticks Attached 7 Days Later
Rabbit A	44	22 R	50 µg	8	82
		22 L		36	
Rabbit B	43	25 R	50 µg	4	91
		18 L		39	
Rabbit C	47	23 R	50 µg	34	78
		24 L		10	
Rabbit D	40	18 R	Control	21	48
		22 L		19	
Rabbit E	45	21 R	Control	21	53
		24 L		24	
Rabbit F	47	24 R	Control	24	51
		23 L		23	
					se 3.25

Significant difference between treatment and control at 5% level.

Replicates	No. of Ticks Attached	No. Moved on Treated Rabbits	No. Moved on Control Rabbits
6	266	49	3

% of ticks on treated sides of treated rabbits - 84
% of ticks on corresponding sides of control rabbits - 51

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*Subsequent applications made at 2-day intervals.

Table 6. Distribution of Previously Attached Male Ticks (Gulf Coast) on Rabbits 7 Days After Initial Application* of 2,6-Dichlorophenol (50 adults/rabbit). Series 2.

Replicate No.	No. of Ticks Attached	Side of Rabbit	Dosage	Ticks/Side 7 Days Later	% of Ticks Attached 7 Days Later
Rabbit A	50	25 R 25 L	50 µg	18 32	64
Rabbit B	48	25 R 23 L	50 µg	3 43	90
Rabbit C	46	25 R 21 L	50 µg	9 37	80
Rabbit D	49	25 R 24 L	Control	24 23	47
Rabbit E	47	25 R 22 L	Control	22 25	53
Rabbit F	46	23 R 23 L	Control	21 23	50
					se 5.44

Significant difference between treatment and control at 5% level.

Replicates	No. of Ticks Attached	No. Moved on Treated Rabbits	No. Moved on Control Rabbits
6	286	48	6

% of ticks on treated sides of treated rabbits - 78
% of ticks on corresponding sides of control rabbits - 50

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*Subsequent applications made at 2-day intervals.

Table 7. Distribution of Previously Attached Male Ticks (Lone Star) on Rabbits 7 Days After Initial Application* of 2,6-Dichlorophenol (50 adults/rabbit). Series 1.

Replicate No.	No. of Ticks Attached	Side of Rabbit	Dosage	Ticks/Side 7 Days Later	% of Ticks Attached 7 Days Later
Rabbit A	48	24 R	50 μg	44	92
		24 L		2	
Rabbit B	47	23 R	50 μg	38	81
		24 L		2	
Rabbit C	49	24 R	50 μg	32	65
		25 L		16	
Rabbit D	47	23 R	Control	23	49
		24 L		23	
Rabbit E	48	23 R	Control	23	48
		25 L		25	
Rabbit F	49	24 R	Control	24	49
		25 L		24	

se 5.7

Significant difference between treatment and control at 5% level.

Replicates	No. of Ticks Attached	No. Moved on Treated Rabbits	No. Moved on Control Rabbits
6	288	43	0

% of ticks on treated sides of treated rabbits - 79
% of ticks on corresponding sides of control rabbits - 49

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*Subsequent applications made at 2-day intervals.

Table 8. Distribution of Previously Attached Male Ticks (Lone Star) on Rabbits 7 Days After Initial Application* of 2,6-Dichlorophenol (50 adults/rabbit). Series 2.

Replicate No.	No. of Ticks Attached	Side of Rabbit	Dosage	Ticks/Side 7 Days Later	% of Ticks Attached 7 Days Later
Rabbit A	38	22 R 16 L	50 μg	35 1	92
Rabbit B	50	26 R 24 L	50 μg	40 10	80
Rabbit C	44	21 R 23 L	50 μg	39 3	89
Rabbit D	48	25 R 23 L	Control	21 23	44
Rabbit E	47	25 R 22 L	Control	27 20	57
Rabbit F	43	21 R 22 L	Control	21 22	49
					se 3.72

Significant difference between treatment and control at 5% level.

Replicates	No. of Ticks Attached	No. Moved on Treated Rabbits	No. Moved on Control Rabbits
6	270	49	2

% of ticks on treated sides of treated rabbits - 87
% of ticks on corresponding sides of control rabbits - 50

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*Subsequent applications made at 2-day intervals.

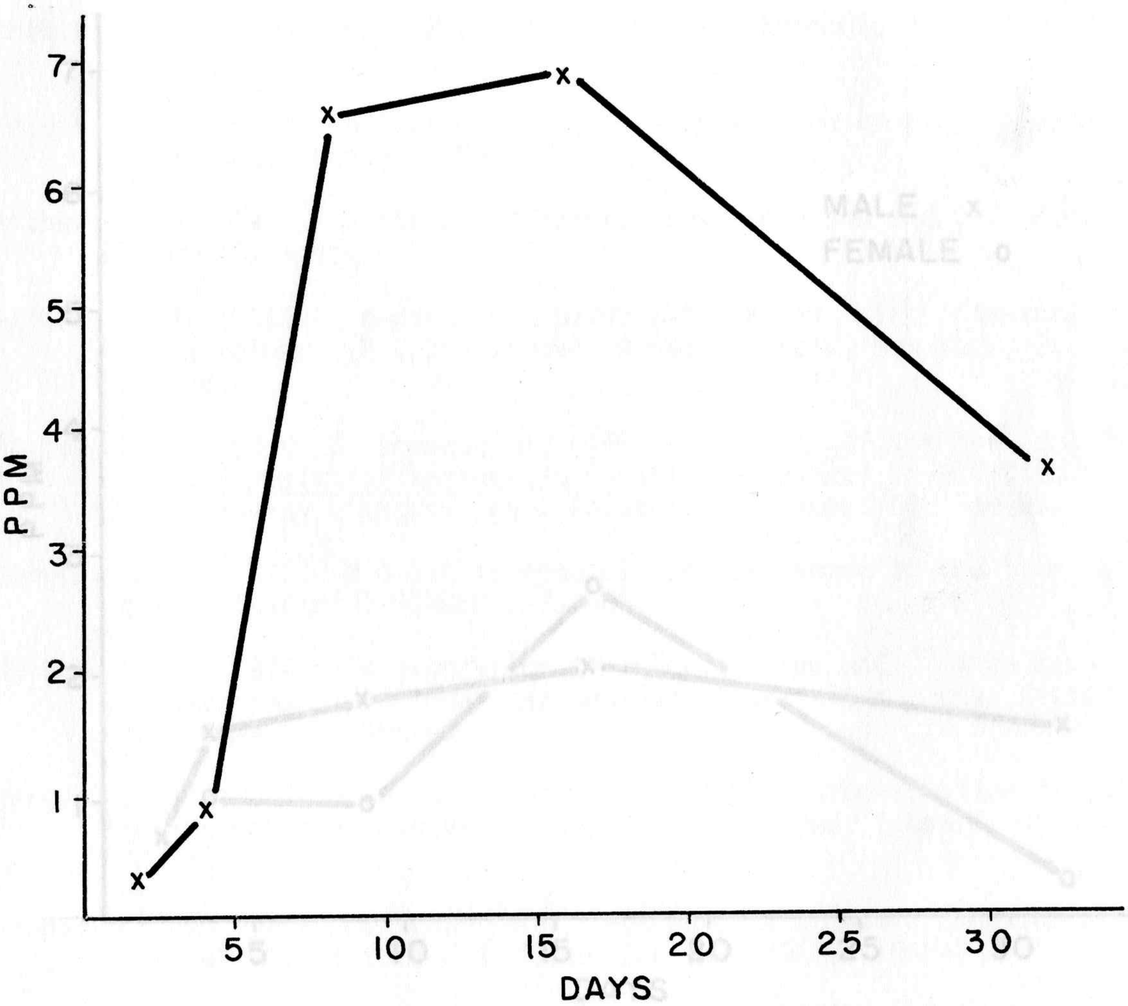

Figure 1. Concentration of 2,6-Dichlorophenol in Unfed Lone Star Adult Female Ticks at Various Times After Emergence.

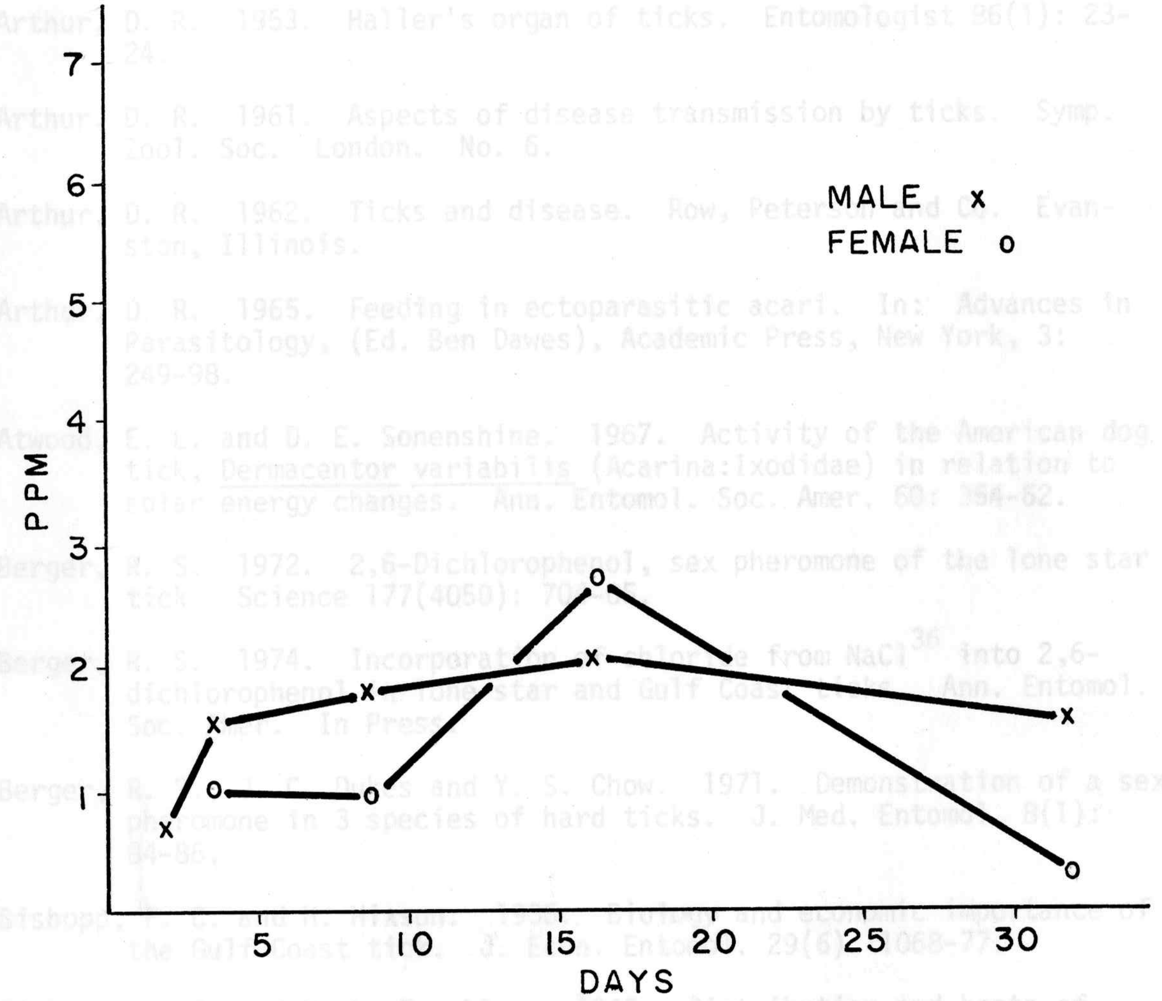

Figure 2. Concentration of 2,6-Dichlorophenol in Unfed Gulf Coast Adult Ticks at Various Times After Emergence.

LITERATURE CITED

Amin, O. M. 1969. Growth of the dog tick, Dermacentor variabilis Say (Acarina:Ixodidae). 1. Growth pattern. J. Med. Entomol. 6(3): 305-16.

Arthur, D. R. 1953. Haller's organ of ticks. Entomologist 86(1): 23-24.

Arthur, D. R. 1961. Aspects of disease transmission by ticks. Symp. Zool. Soc. London. No. 6.

Arthur, D. R. 1962. Ticks and disease. Row, Peterson and Co. Evanston, Illinois.

Arthur, D. R. 1965. Feeding in ectoparasitic acari. In: Advances in Parasitology, (Ed. Ben Dawes), Academic Press, New York, 3: 249-98.

Atwood, E. L. and D. E. Sonenshine. 1967. Activity of the American dog tick, Dermacentor variabilis (Acarina:Ixodidae) in relation to solar energy changes. Ann. Entomol. Soc. Amer. 60: 354-62.

Berger, R. S. 1972. 2,6-Dichlorophenol, sex pheromone of the lone star tick. Science 177(4050): 704-05.

Berger, R. S. 1974. Incorporation of chloride from $NaCl^{36}$ into 2,6-dichlorophenol in lone star and Gulf Coast ticks. Ann. Entomol. Soc. Amer. In Press.

Berger, R. S., J. C. Dukes and Y. S. Chow. 1971. Demonstration of a sex pheromone in 3 species of hard ticks. J. Med. Entomol. 8(1): 84-86.

Bishopp, F. C. and H. Hixson. 1936. Biology and economic importance of the Gulf Coast tick. J. Econ. Entomol. 29(6): 1068-77.

Bishopp, F. C. and H. L. Trembley. 1945. Distribution and hosts of certain North American ticks. J. Parasitol. 31: 1-54.

Brennan, J. M. 1945. Field investigations pertinent to Bullis Fever: the lone star tick, Amblyomma americanum (Linnaeus, 1858). Notes and observations from Camp Bullis, Texas. Texas Rept. Biol. Med. 3: 205-06.

Chinery, W. A. 1973. The nature and origin of the "cement" substance at the site of attachment and feeding of adult Haemaphysalis spinigera (Ixodidae). J. Med. Entomol. 10(4): 355-62.

Clymer, B. C., D. E. Howell and J. A. Hair. 1970. Environmental alteration in recreational areas by mechanical and chemical treatment as a means of lone star tick control. J. Econ. Entomol. 63: 504-09.

Cooley, R. A. and G. M. Kohls. 1944. The genus Amblyomma (Ixodidae) in the United States. J. Parasitol. 30: 77-111.

Cooney, J. C. and K. L. Hays. 1972. The ticks of Alabama (Ixodidae: Acarina). Agr. Exp. Sta. Auburn Univ. Bull. 426. 40p.

Douglas, J. R. 1943. The internal anatomy of Dermacentor andersoni stiles. Univ. Calif. Publ. Entomol. 7: 207-72.

Drummond, R. O., J. C. Medley and O. H. Graham. 1966. Engorgement and reproduction of lone star ticks, Amblyomma americanum (L.) treated with gamma radiation. Int. J. Radiat. Biol. 10(2): 183-88.

Drummond, R. O. and T. M. Whetstone. 1970. Oviposition of the Gulf Coast tick. J. Econ. Entomol. 63(5): 1547-51.

Drummond, R. O., T. M. Whetstone and W. J. Gladney. 1971. Oviposition of the lone star tick. Ann. Entomol. Soc. Amer. 64(1): 191-94.

Feldman-Muhsam, B. 1973. Autogeny in soft ticks of the genus Ornithodorus (Acari:Argasidae). J. Parasitol. 57: 630-34.

Galun, R. and S. H. Kindler. 1968. Chemical basis of feeding in the tick Orinithodorus tholozani. J. Insect Physiol. 14: 1409-21.

Garcia, R. 1962. Carbon dioxide as an attractant for certain ticks (Acarina:Argasidae and Ixodidae). Ann. Entomol. Soc. Amer. 55: 605-06.

Gladney, W. J. 1971. Mate seeking by female Amblyomma maculatum (Acarina:Ixodidae) on a bovine. Nature. London. 232: 401-02.

Gladney, W. J. and R. O. Drummond. 1970. Mating behavior and reproduction of the lone star tick, Amblyomma americanum. Ann. Entomol. Soc. Amer. 63(4): 1036-39.

Gladney, W. J. and R. O. Drummond. 1971. Spermatophore transfer and fertilization of lone star ticks off the host. Ann. Entomol. Soc. Amer. 64(2): 378-81.

Gladney, W. J., R. O. Drummond, T. M. Whetstone and S. E. Ernst. 1970. Effect of age on the attachment rate of the parasitic stages of the lone star tick, *Amblyomma americanum* (L.) (Acarina:Ixodidae) in the laboratory. J. Med. Entomol. 7(1): 92-95.

Gladney, W. J., O. H. Graham, J. L. Trevino and S. E. Ernst. 1973. *Boophilus annulatus*, effect of host nutrition on development of female ticks. J. Med. Entomol. 10(2): 123-30.

Gladney, W. J. and J. E. Wright. 1971. Effect of photoperiod on the rate of attachment of *Amblyomma americanum* (Acarina:Ixodidae). Ann. Entomol. Soc. Amer. 64: 303-05.

Granett, P. and B. Sacktor. 1947. Testing tick repellents and observations of phototropic effects. J. Econ. Entomol. 40: 259-63.

Gregson, J. D. 1953. Review of recent contributions to the study of tick paralysis in North America. Proc. VI Int. Congr. for Microbiology (Rome).

Gregson, J. D. 1960. Morphology and functioning of the mouthparts of *Dermacentor andersoni* Stiles. Acta. Trop. 17: 48-79.

Gregson, J. D. 1966. Ticks. In: Insect Colonization and Mass Production. C. N. Smith, Ed., Academic Press, New York. p. 49-72.

Hindle, E. and G. Merriman. 1912. The sensory perceptions of *Argas persicus* (Oken). Parasitology p. 203-16.

Hixson, H. 1940. Field biology and environmental relationships of the Gulf Coast tick in Southern Georgia. J. Econ. Entomol. 33(1): 178-89.

Hoch, A. L., R. W. Barker and J. A. Hair. 1971. Measurement of physical parameters to determine suitability of modified wood-lots as lone star tick habitat. J. Med. Entomol. 8(6): 725-30.

Hooker, W. A., F. C. Bishopp and H. P. Wood. 1912. The life history and bionomics of some North American ticks. U.S.D.A. Bur. Entomol. Bull. 106, 239p.

Jacobson, M. 1972. Insect Sex Pheromones. Academic Press, New York and London, 382p.

Knipling, E. F. and J. U. McGuire, Jr. 1966. Population models used for insect control. Agr. Inf. Bull. No. 308. U.S. Dept. of Agr.

Lancaster, J. L., Jr. 1955. Biology and seasonal history of the lone star tick in northwest Arkansas. J. Econ. Entomol. 48(3): 295-97.

Lancaster, J. L., Jr. 1956. Some effects of varying temperature and humidity on the lone star tick (Amblyomma americanum Linnaeus). Proc. 10th Int. Congr. Entomol. 3: 849-52.

Lancaster, J. L., Jr. and H. L. MacMillan. 1955. The effects of humidity on the lone star tick. J. Econ. Entomol. 48: 338-39.

Lees, A. D. 1946. The water balance in Ixodes ricinus (L.) and certain other species of ticks. Parasitology 37(1): 1-20.

Lees, A. D. 1948. The sensory physiology of the sheep tick, Ixodes ricinus (L.). J. Exp. Biol. 25(2): 145-207.

Lees, A. D. and A. Milne. 1951. The seasonal and diurnal activities of individual sheep ticks, Ixodes ricinus (L.). J. Parasitol. 41: 189-208.

Loomis, E. C. 1961. Life histories of ticks under laboratory conditions (Acarina:Ixodidae and Argasidae). J. Parasitol. 47(1): 91-99.

Moorhouse, D. E. and R. T. Tatchell. 1966. The feeding process of the cattle tick, Boophilus microplus (Canestrini): A study in host-parasite relations. Part 1. Attachment to the host. Parasitology 56: 623-32.

Oliver, J. H. 1974. Reproduction in ticks. J. Med. Entomol. 11(1): 26-34.

Reik, R. F. 1959. Studies on the reactions of animals to infestations with ticks. IV. The protein components of the tick extracts. Aust. J. Agr. Res. 10: 604-13.

Robinson, L. E. 1926. The genus Amblyomma. Vol. 4. In: Nuttall et al.: Ticks, a monograph of Ixodoidea. Cambridge Univ. Press. 302p.

Ryan, E. P. 1966. Pheromone: Evidence in a decapod crustacean. Science 151: 340-41.

Sacktor, B., M. Hutchinson and P. Granett. 1948. Biology of the lone star tick in the laboratory. J. Econ. Entomol. 41(2): 296-301.

Sauer, J. R. and J. A. Hair. 1971. Water balance in the lone star tick (Acarina:Ixodidae): The effects of relative humidity and temperature on weight changes and total water content. J. Med. Entomol. 8(5): 478-85.

Sauer, J. R. and J. A. Hair. 1972. The quantity of blood ingested by the lone star tick (Acarina:Ixodidae). Ann. Entomol. Soc. Amer. 65(5): 1065-68.

Semtner, P. J., R. W. Barker and J. A. Hair. 1971. The ecology and behavior of the lone star tick (Acarina:Ixodidae). 11. Activity and survival in different ecological habitats. J. Med. Entomol. 8: 719-25.

Semtner, P. J. and J. A. Hair. 1973. The ecology and behavior of the lone star tick (Acarina:Ixodidae). V. Abundance and seasonal distribution in different habitat types. J. Med. Entomol. 10(6): 618-28.

Shorey, H. H., L. K. Gaston and C. A. Saario. 1967. Sex pheromones of Noctuid moths. XIV. Feasibility of behavioral control by disrupting pheromone communication in cabbage loopers. J. Econ. Entomol. 60: 1541-45.

Smith, C. N., N. M. Cole, I. H. Gilbert and H. K. Gauch. 1954. Field tests with tick repellents--1949, 1950 and 1952. J. Econ. Entomol. 47: 13-19.

Snodgrass, R. E. 1948. The feeding organs of Arachnida, including mites and ticks. Smithsonian Misc. Coll. Vol. 110 No. 10. Wash. D.C. Smithsonian Inst. (Publ. No. 3944). 93p.

Sonenshine, D. E. and E. L. Atwood. 1967. Dynamics of feeding of the American dog tick Dermacentor variabilis (Acarina:Ixodidae). Ann. Entomol. Soc. Amer. 60(2): 362-73.

Sonenshine, D. E., E. L. Atwood and J. T. Lamb. 1966. Ecology of ticks transmitting Rocky Mountain spotted fever in a study area in Virginia. Ann. Entomol. Soc. Amer. 59: 1234-64.

Sonenshine, D. E. and G. F. Levy. 1971. The ecology of the lone star tick Amblyomma americanum (L.) in two contrasting habitats in Virginia (Acarina:Ixodidae). J. Med. Entomol. 8: 623-35.

Sonenshine, D. E. and J. A. Tigner. 1969. Oviposition and hatching in two species of ticks in relation to moisture deficit. Ann. Entomol. Soc. Amer. 62: 628-40.

Tatchell, R. J. 1967. Salivary secretion in the cattle tick as a means of water elimination. Nature. London. 213: 940-41.

Webb, J. E., Jr., J. C. Clark and A. L. Adams. 1956. Studies on the lone star tick. Texas J. of Sci. 8: 399-404.

Wilson, J. G., D. R. Kinzer, J. R. Sauer and J. A. Hair. 1972. Chemoattraction in the lone star tick (Acarina:Ixodidae): 1. Response of different developmental stages to carbon dioxide administered via traps. J. Med. Entomol. 9: 245-52.

Woolley, T. A. 1972. Some sense organs of ticks as seen by scanning electron microscopy. Trans. Amer. Microsc. Soc. 9(1): 35-47.

Wright, J. E. 1971. Response of ovipositing Amblyomma maculatum Koch (Acarina:Ixodidae) to photoperiod. J. Med. Entomol. 8(5): 529-31.